GUIDELINE C-17

Non-Incineration Technologies for Treatment of Biomedical Waste (Procedures for Microbiological Testing)

Legislative Authority:

Environmental Protection Act, Part V,

Sections 19 and 27; Part XVII, Section 197

Responsible Director:

Director, Standards Development Branch
Ministry of the Environment

Latest Revision Date: October 2002

Ministry of the Environment

Ontario

Synopsis

This guideline applies to non-incineration technologies used in the treatment of biomedical waste in Ontario, including both new and existing facilities of any size.

On December 18, 2001, the Ministry of the Environment posted on the Environmental Bill of Rights Registry [EBR Registry Number RA01E0023], proposed changes to Regulation 347 which would add a new, comprehensive definition ofb iomedical wastes. This guideline was written in anticipation of such changes.

Non-incineration technologies include all technologies that are not included in Guideline A-1, (Combustion, Air Pollution Control and Monitoring Requirements for Biomedical Waste Incineration in Ontario, October 2002).

The purpose of this guideline is to ensure that non-incineration technologies are capable of sterilizing or adequately disinfecting biomedical waste. This is verified at the commissioning of the equipment at a new site, and further verified at regular intervals by testing the efficacy of the operating equipment. The verification involves strict procedures and the test results are reviewed before suitable disposal of the treated biomedical waste may proceed.

This guideline will be applied through conditions on certificates of approval for new or upgraded biomedical waste treatment in accordance with the requirements of the *Environmental Protection Act*, Part V, Section 27, and Part II, Section 9.

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1.0 Introduction

Under Ontario Regulation 558/00 (amending Regulation 347, in effect March 31, 2001), residual wastes derived from the treatment of biomedical waste remain biomedical waste and must be managed as such. If, however, biomedical waste is treated by means of non-incineration treatment technologies which comply with the applicable procedures described in this guideline, the resultant waste will be considered to be non-hazardous, treated biomedical waste.

The purpose of this document is to provide information regarding the preparation of detailed procedures for the microbiological testing of non-incineration technologies for the treatment of biomedical wastes.

It is important to ensure that equipment is capable of sterilizing or adequately disinfecting biomedical wastes. To do so, the procedures must include the monitoring of relevant operating conditions (such as minimum temperature, minimum pressure, residence time, chemical concentrations, and pH) which ensure that appropriate sterilization or high level disinfection is achieved on a continuing basis. It is also important to be able to show that the equipment continues to provide this level of sterilization/disinfection during normal operations.

As a result, two procedures are required to provide assurance that the technology is operating properly:

- one procedure for commission testing on equipment in use on and after March 31, 2001
- a second procedure for on-going verification testing for operating equipment.

2.0 Guideline Limits

2.1 Levels of Treatment

There are four levels of treatment defined by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO):

Level 1 – Low Level Disinfection:

Inactivation of most vegetative bacteria, fungi, and some viruses. This level of treatment does not inactivate mycobacteria (bacteria causing tuberculosis) and bacterial spores. Furthermore, this level of treatment is inadequate for biomedical waste treatment and is not recommended.

Level 2 – Intermediate Level Disinfection:

Inactivation of all mycobacteria, viruses, fungi and vegetative bacteria. It does not include the inactivation of bacterial spores. The CDC defines this as the destruction of all microorganisms except high numbers of bacterial spores. These two definitions are essentially equivalent. Tests for intermediate level disinfection mustsh ow that a 6 log (logarithm to the base 10) reduction of the microorganism most resistant to the treatment is attained. This level does not include inactivation of bacterial spores which are required in Level 3 and Level 4.

Level 3 – High Level Disinfection:

The killing of all microbial life forms present in a medical waste load as evidenced by the inactivation of surrogate pathogens (bacterial spores) having death curves similar to the most resistant human pathogens. Such surrogate pathogens may not be the forms most resistant to a particular treatment process but are similar in resistance to most human pathogens found in biomedical waste. This level of treatment requires the inactivation of a specific quantity of a resistant surrogate pathogen, thus assuring that the waste is treated to reduce the quantity of infectious agents present in the waste stream to a level that does not present a significant risk to human health or the environment. A minimum of 4 log reduction of spores of either *B. stearothermophilus* or *B. subtilis* by thermal inactivation technologies or by chemical treatment is accepted as indicating high level and intermediate level disinfection. A 4 log 10 reduction is equivalent to a 99.99 % reduction in spores.

Level 4 – Sterilization:

The killing of all microbial life forms as indicated by complete inactivation of specific concentrations of those organisms recognized as most resistant to the treatment process. Sterilization is evidenced by a minimum 6 log reduction in spores of *B. stearothermophilus*.

2.2 Regulated Reduction Levels

Table 1 summarizes the reduction levels required under this Guideline.

The high level of disinfection (level 3) is the minimum level of disinfection accepted. Where the technology is proven to exceed these minimum standards, the standard matches what the technology is capable of achieving. Steam sterilization, such as autoclave (gravity assisted or steam assisted), external steam agitation (ESA) and hydroclave technologies, have been proven to attain the sterilization level (level 4) under normal operation.

For steam sterilization processes, the tests are required to be done only on the heat resistant spores of *Bacillus Stearothermophilus*.

High disinfection level (level 3) should be met for all other treatmentp rocesses. Either *B. stearothermophilus* or *B. subtilis* can be used for non thermal processes (i.e. chemical,m icrowave and macrowave technologies).

Table 1: Regulated Reduction Levels

Process -- Technology	**Reduction of *B.stearothermophilus* spores (Sterilization - level 4)**	***Reduction of B.stearothermophilus* or *B. subtilis* spores (High Level Disinfection - level 3)***	***Comments***
Steam sterilization	*6 log 10*	-	*Follow procedure 3.1*
Chemical	-	*4 log 10*	*Follow procedure 3.2*
Microwave	-	*4 log 10*	*Follow procedure 3.3*
Macrowave	-	*4 log 10*	*Follow procedure 3.4*

3.0 Commission Testing Procedure

The following are general guidelines for the site operator of non-incineration equipment when developing a procedure for commission testing the equipment. Additional specific guidelines are listed in sections 3.1 to 3.4 for specific treatment processes.

a) Microbiological tests shall be performed under actual operating conditions of the treatment process at full operational load conditions.

b) Waste shall be representative of the actual biomedical waste to be treated on an on-going basis.

c) If commercial spore strips, suspensions or self contained vials are used in the testing, the expiry date must be noted and not exceeded.

d) Test samples shall be kept at 1°C to 4°C after the sample is taken during transportation to the testing lab and during storage at the lab unless they are analyzed on site. Analysis shall be started within 24 hours of receipt using standard microbiological techniques acceptable to the Ministry of the Environment.

3.1 Commission Testing for Steam Sterilization

The following are guidelines for sterilization (level 4) commission testing procedures for steam sterilization processes (including autoclave, hydroclave, external steam agitation):

a) Commissioning tests for steam sterilization should be performed with B. stearothermophilus spores; a 6 log reduction of these spores must be achieved to prove that the technology sterilizes the biomedical waste. The ATCC number of spores to be used in the testing must be specified.

b) The equipment, materials and reagents to be used in the commission testing must be specified.

c) The following methods must be presented in detail:

1. the method of organism procurement, identification, storage and preparation;
2. the method of placing the organisms in the biomedical waste load at the points which ensure that the entire load has been properly treated;
3. the method of recovering any surviving organisms;

4. the method of recording operational parameters such as the time, temperature and pressure during the testing.

3.2 Commission Testing for Chemical Technologies

The following are guidelines for high level disinfection (level 3) commission testing procedures for chemical disinfection technologies:

a) Commissioning tests for chemical disinfection technologies should be performed with *B. stearothermophilus* or *B. subtilis* spores. A 4 log reduction of either of these spores must be achieved to prove that the technology provides high level disinfection of biomedical waste. The ATCC number of the spores to be used in the testing must be specified.

b) The equipment, materials and reagents to be used in the commission testing must be specified.

c) The following methods must be presented in detail:

1. The method ofo rganism procurement, identification, storage and preparation;
2. the method of placing the organisms in the biomedical waste load at the points which ensure that the entire load has been properly treated;
3. the method of recovering the surviving organisms;
4. the method of recording operational parameters such as the residence time, temperature and reagent concentrations during the testing.

d) Provision must be made for the rapid inactivation of any residual disinfectant or other microbiological inhibitor in test samples at the time the sample is taken.

3.3 Commission Testing for Microwave Technology

The following are guidelines for high level disinfection (level 3) commission testing procedures for the microwave technology:

a) Commissioning tests for the microwave technology should be performed with *B. stearothermophilus* or *B. subtilis* spores; a 4 log reduction of either of these spores must be achieved to prove that the technology provides high level disinfection of the biomedical waste. The ATCC number of the spores to be used in the testing must be specified.

b) The equipment, materials and reagents to be used in the commission testing must be specified.

c) The following methods must be presented in detail:

1. the method of organism procurement, identification, storage and preparation;
2. the method of placing the organisms in the biomedical waste load at the points which ensure that the entire load has been properly treated;
3. the method of recovering any surviving organisms;
4. the method of recording operational parameters such as the retention time in the heated portion of the technology, temperature of the load during treatment, and microwave power input during treatment.

3.4 Commission Testing for Macrowave Technology

The following are guidelines for high level disinfection (level 3) commission testing procedures for the macrowave (short wave radio frequency) technology:

a) Commissioning tests for the macrowave technology should be performed with B. stearothermophilus or B. subtilis spores; a 4 log reduction of either of these spores must be achieved to prove that the technology provides high level disinfection of biomedical waste. The ATCC number of the spores to be used in the testing must be specified.

b) The equipment, materials and reagents to be used in the commission testing must be specified.

c) The number of treatment containers to be tested must be specified.

d) The following methods must be presented in detail:

1. the method of organism procurement, identification, storage and preparation;
2. the method of placing the organisms in the biomedical waste treatment container at the points which ensure that the entire load has been properly treated;
3. the method of recovering the surviving organisms;
4. the method of recording operational parameters such as the retention time at temperatures above 90°C;te mperature of the load during treatment at the top, middle and bottom of the treatment containers; and macrowave power input during treatment.

4.0 Operational Requirements

a) All procedures specified by the manufacturer of the equipmentm ust be followed for each treated load. This includes visually monitoring, recording (and/or datalogging) and maintaining a log book of key operation parameters during the treatment. The logged information will include date/time, load identification and key operational parameters during the treatment, as defined in the manufacturer manual or recommended by the Ministry (for example, in the case of steam sterilization, these will include pressure and sterilization time).

b) Maintenance must be completed as per the manufacturers advice.

c) Any failure in the system that places in doubtth e efficacy of the treatment requires immediate action for the management of the untreated load.

d) No waste shall leave the site as non-hazardous waste if there is a system failure or if the prescribed key operation parameters were not maintained.

5.0 Procedure For Verification Testing

5.1 Verification Testing for Steam Sterilization

Verification testing is required to ensure that the technology continues to achieve sterilization (Level 4). The following are guidelines for the site operator of non-incineration equipment when developing a procedure for verification testing of steam sterilization technologies (including "autoclaving", "hydroclaving" and "external steam agitation"):

a) At least every six (6) days of operation, the Company shall undertake verification testing ofa representative batch of biomedical waste, using a biological indicator.

b) Reference spore strips, suspensions or self-contained vials of viable spores are to be subjected to the typical treatment process. A biological indicator with a population of at least one million spores (1×10^6) must be used. The co-treatment of the reference spores and waste mustf ollow a clear documented process. The reference spores are then tested to ensure the desired level of treatment (sterilization, i.e. 6 log 10 reduction) of the waste load has been achieved. Tests for spores in the actual waste being treated is not required.

c) The reference spore samples associated with the testing shall be cultured and monitored and tested in accordance with the specifications of the manufacturers.

d) Test results must confirm 6 log 10 reduction of spores.

e) Following an unsuccessful verification test, no further loads are allowed to leave the treatment site.

f) If any load fails verification testing, that load and the previous loads stored on site are considered untreated. The reason for the failure must be assessed and the problem corrected and documented. All loads that had not left the site shall be re-treated before leaving the site. Loads can only leave the site as non-hazardous waste [treated biomedical waste] after a successful verification of 6 log 10 reduction of spores.

Other new technologies that demonstrate that they consistently achieve sterilization level (i.e. 6 log 10 attenuation) may be considered for verification testing similar to the steam sterilization procedure. Proponents should contact the director of the Standards Development Branch for consideration.

5.2 Verification Testing for Chemical, Micro/Macro Wave Treatments, and Other New Technologies

Verification testing is required to ensure that the technology continues to achieve high level disinfection (Level 3). The following are guidelines for the site operator of non-incineration equipment when developing a procedure for verification testing for chemical,m icrowave and macrowave technologies and other new technologies:

a) At least every six (6) days of operation, the Company shall undertake verification testing ofa representative batch of biomedical waste, using a biological indicator.

b) Reference spore strips, suspensions or self-contained vials of viable spores are to be subjected to the typical treatment process. A biological indicator with a population of at least ten thousand spores (1×10^4) must be used. The co-treatment of the reference spores and waste mustf ollow a clear documented process.T he reference spores are then tested to ensure the desired level of treatment (high level disinfection, i.e. 4 log 10 reduction) of the waste load has been achieved. Tests for spores in the actual waste being treated is not required.

c) The reference spore samples associated with the testing shall be cultured and monitored and tested in accordance with the specifications of the manufacturers.

d) Test results must confirm 4 log10 reduction of spores.

e) Following a successful verification test, all loads treated subsequently must be adequately stored on site until the next successful verification test. Only then will the tested load and the loads treated since the previous successful verification test be allowed to leave the treatment site for disposal.

f) If any load fails verification testing, that load and the previous loads stored on site are considered untreated. The reason for the failure must be assessed and the problem corrected and documented. All loads that had not left the site shall be re-treated before leaving the site. Loads can only leave the site as non-hazardous waste [treated biomedical waste] after a successful verification of 4 log 10 reduction of spores.

6.0 Records And Reports

a) A reporto n commission testing shall be prepared and retained.

b) The complete commission testing procedure for the equipment must be retained.

c) A reporto n each occurrence of verification testing (including recording and charts for the operation parameters) shall be prepared and retained for 2 years.

d) The complete verification testing procedure for the equipment, which is to contain similar information to the commissioning procedure, and including any modifications made to the procedure, must be retained..

e) A record of all maintenance shall be prepared and maintained.

f) Unless specified otherwise, any report or other document which is to be retained, shall be retained for the life of the equipment.

g) A copy ofa ny reporto r other document which is to be retained, shall be available on request to the Ministry of the Environment.

7.0 Approvals

Part V of the *Environmental Protection Act* requires that a proponent ofa commercial hazardous waste treatmentf acility apply to the Ministry of the Environment for approval to install and operate a waste management facility. In the review of the application and in the conditions of the certificate of approval, the Ministry will incorporate conditions requiring that the procedures in this guideline be implemented at the facility.

For on-site waste management, the Ministry does not typically require that the facility obtain waste management approvals for those activities. Biomedical waste generating facilities using non-incineration technologies and following the requirements specified in this guideline, would produce residual wastes which are considered to be treated biomedical waste as defined in Regulation 347.

8.0 Definitions

Autoclave:

Steam autoclave treatment combines moisture, heat and pressure to inactivate microorganisms. All steam autoclaves are constructed with a metal chamber to withstand the increased pressure/temperature. Autoclaves come in two basic varieties, gravity displacement and prevacuum autoclaves. The size of the devices may vary from bench top models to large commercial models which can treatm ore than a ton of waste per cycle.

Chemical Disinfection Technologies:

Chemical disinfection is achieved by using sodium hypochlorite solution to kill microorganisms. The process requires that the biomedical waste units (bags, boxes or other type of containers) be shredded. Disinfection is achieved when there is a reduction of 4 log 10 (99.99% reduction) in the spores of *B. stearothermophilus* or *B. subtilis*. Other chemicals such as chlorine derivatives, ozone or enzymes can be used in chemical disinfection.

Disinfection:

Disinfection refers to a level of destruction or inactivation of pathogen bacteria. Disinfection levels can range from low level to high level, to sterilization. Sterilization is the highest level of disinfection in biomedical treatment.

Steam sterilization:

Steam sterilization includes autoclave, external steam agitation (ESA), hydroclave (TM), and similar autoclave processes where steam, heat and pressure are used. The additional conditions for ESA is that the waste is mixed and broken down (not necessarily shredded) by internal mixing arms. As a general rule, the destruction of pathogens is more efficient under these conditions, because of easier and better steam penetration in the waste.

Macrowave Technology:

Biomedical wastes are heated in a chamber for a minimum of 30 minutes at 95 C using macrowaves in the region of 64 MHz.

Microwave Technology:

Biomedical wastes are heated for a minimum of 30 minutes at 95 C using microwaves in the vicinity of 2,450 MHz.

Sterilization:

Sterilization refers to a level 4 treatment and involves the killing of all microbial life forms to a level of 6 log 10 or higher, meaning that a least 99.9999 % of the original spores of *B. stearothermophilus* have been destroyed in the waste, or that only one spore or less has survived the treatment from a population of one million spores.

9.0 Abbreviations

ATCC	American Type Culture Collection
4 log 10	Defined as a 4 decade reduction or a 0.0001 survival probability in a microbial population; i.e. a 99.99 % reduction
6 log 10	Defined as a 6 decade reduction or a 0.000001 survival probability in a microbial population; i.e. a 99.9999 % reduction
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization